PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12437050/ejh/JEH/RBR	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. **PCT/AU2004/000511**	International filing date *(day/month/year)* 16 April 2004	Priority date *(day/month/year)* 17 April 2003
International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[7] C12N 15/62, 7/01, 15/63		
Applicant HEPGENICS PTY LTD et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of **8** sheets, as follows:
      - [ ] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [X] Box No. VIII Certain observations on the international application

Date of submission of the demand 17 February 2005	Date of completion of the report 23 August 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **TERRY MOORE** Telephone No. (02) 6283 2632

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/AU2004/000511

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1 (b))

   ☐ publication of the international application (under Rule 12.4)

   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

   ☐ the international application as originally filed/furnished

   ☒ the description:

   pages **1-57** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☒ the claims:

   pages as originally filed/furnished
   pages* as amended (together with any statement) under Article 19
   pages* **58-65** received by this Authority on **17 February 2005** with the letter of 27 January 2005
   pages* received by this Authority on with the letter of

   ☒ the drawings:

   pages **1-12** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-45	YES
	Claims		NO
Inventive step (IS)	Claims	1-45	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-45	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The specification discloses a fusion construct comprising a heterologous peptide of interest fused with the C terminal region, or S domain of the large envelope protein of a hepadnavirus. The disclosed constructs can assemble with the S protein into the viral envelope of virus like particles (VLPs) where the heterologous protein will be located on the surface of the particle.

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 Proc Natl Acad Sci USA 92, 6259-63
D2 J Biol Chem 267(3), 1953-61

Novelty and Inventive Step

D1 discloses L protein fusions where the preS region of one hepadnavirus is fused with the S domain, or particle associating portion, of a second hepadnavirus. The specific hepadnaviruses exemplified are duck HBV and heron HBV, see figure 4 and the protein fusions are assembled in infectious virus.

However, D1 relates to the preparation of infectious virus in contrast to the applicant's claimed invention, which relates to the preparation of virus like particles (VLPs). In addition, with respect to claims 13-30 the POI is a preS region of a hepadnavirus, a feature that is excluded from the scope of claims 13-30.

As such D1 does not appear to be relevant to the novelty or inventive step of the claims.

D2 discloses L protein fusions with the signal peptide of chicken lysozyme. These fusion proteins provide the signal peptide of chicken lysozyme as the protein of interest and the full length L protein as the particle-associating portion of the L protein.

However the D2 L protein is derived from hepatitis B virus (HBV), a non-avian hepadnavirus where L proteins are excluded during VLP assembly. As such D2 does not appear to deprive the claims of novelty or an inventive step.

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The specification lacks clarity. Page 41, line 5 states that pCDL-E2.465 comprises DHBV L aa 1-4, however it appears from a reading of the specification that this should read DHBV aa 1-45.

Claims 1, 4 and 11 are not fully supported by the description because the claims are not restricted to avian hepadnaviruse L proteins and simply define any hepadnavirus L protein. The description discloses fusion peptides derived from the L protein of avian hepatitis viruses and illustrates their ability to assemble into VLPs with the S protein. As such the specification provides support for extending this principle to other avian hepadnaviruses that presumably follow the same assembly pathway. However, this does not provide support for fusion peptides comprising the L protein of non-avian hepadnaviruses. As confirmed by the applicant in their response to the ISO, L proteins from non-avian hepadnaviruses do not assemble into VLPs in association with the S protein as is the case with avian hepadnaviruses.

Claims 31 to 39 are not fully supported by the description. The description discloses recombinant constructs comprising sequences encoding the DHBV L protein wherein a cloning site has been introduced into a specific location in the DHBV L protein sequence. In contrast the claims simply define a construct comprising a cloning site at any location in the L protein sequence. As such the claims include within their scope constructs where cloning sites are naturally occurring rather than introduced and where cloning sites are not introduced at the location described in the specification.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/AU2004/000511

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] in written format

   [ ] in computer readable form

   c. time of filing/furnishing

   [X] contained in the international application as filed

   [ ] filed together with the international application in computer readable form

   [ ] furnished subsequently to this Authority for the purposes of search and/or examination

   [ ] received by this Authority as an amendment* on

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Replacement sheets
Claims:

1. A virus-like particle (VLP) comprising i) a fusion polypeptide comprising a polypeptide of interest (POI) and a particle-associating portion of a hepadnavirus large envelope polypeptide (L) or a functional derivative or homolog thereof and ii) a hepadnavirus small envelope (S) polypeptide or a functional derivative or homolog thereof.

2. The VLP of claim 1 comprising a fusion polypeptide comprising a polypeptide of interest (POI) and a particle-associating portion of a large envelope polypeptide (L) of an avian hepadnavirus, such as duck hepatitis B virus (DHBV), or a functional derivative thereof.

3. The VLP of claim 1 or 2 comprising a fusion polypeptide comprising a polypeptide of interest (POI) and a particle-associating portion of a large envelope polypeptide (L) of an avian hepadnavirus, such as duck hepatitis B virus (DHBV), or a functional derivative thereof and ii) a small envelope (S) polypeptide of an avian hepadnavirus, such as DHBV, or a functional derivative thereof.

4. The VLP of claim 1 or 2 wherein the particle-associating portion of L comprises at least the S domain of L, or the S domain of L minus the TM1 domain, or a functional derivative thereof.

5. The VLP of claim 1 or 2 wherein the POI is located in the pre-S domain of L or at the amino terminal side of the S domain of L, or the S domain minus the TM1 domain of L.

6. The VLP of claim 1 or 2 wherein the L polypeptide comprises an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or a functional derivative thereof or comprises an amino acid sequence having at least

50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.

7. The VLP of claim 1 or 2 wherein the particle-associating portion of L polypeptide comprises an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or a functional derivative thereof comprising an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.

8. The VLP of claim 1 or 2 wherein the particle-associating portion of L-polypeptide comprises or consists essentially of amino acids 24 to 107 of SEQ ID NO: 9 or an amino acid sequence having at least 50% similarity thereto.

9. The VLP of any one of claims 1 to 9 wherein the L polypeptide is a DHBV L polypeptide or functional derivative thereof.

10. The VLP of claim 1 or 2 wherein said L polypeptide or particle-associating portion thereof is encoded by a sequence of nucleotides substantially as set forth in SEQ ID NO: 6 or SEQ ID NO: 8 or having at least about 50% similarity to SEQ ID NO: 6 or SEQ ID NO: 8 or a contiguous sequence of nucleotides capable of hybridizing to a complementary form of SEQ ID NO: 6 or SEQ ID NO: 8 under hybridisation conditions of medium stringency.

11. The VLP of claim 1 or 2 wherein the L polypeptide further comprises a signal sequence.

12. An isolated or recombinant polypeptide for use in the assembly of a VLP, comprising a polypeptide of interest (POI) and at least a particle-associating portion of a large envelope polypeptide (L) of an avian hepadnavirus, such as DHBV, or a functional derivative thereof, wherein the POI is not a pre-S region of an avian hepadnavirus.

13. A recombinant polypeptide capable of assembling into a VLP when expressed in a

cell comprising a polypeptide of interest (POI) and at least a particle-associating portion of a large envelope polypeptide (L) of an avian hepadnavirus such as DHBV or a functional derivative thereof, wherein the POI is not a pre-S region of an avian hepadnavirus.

14. The polypeptide of claim 12 or 13 wherein the particle-associating portion of L comprises at least the S domain of L, or the S domain of L minus the TM1 domain, or a functional derivative thereof.

15. The polypeptide of claim 12 or 13 wherein the POI is located in the pre-S domain of L or at the amino terminal side of the S domain of L, or the S domain minus the TM1 domain of L.

16. The recombinant or isolated polypeptide of claim 12 or 13 wherein the L polypeptide comprises an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or a functional derivative thereof or comprises an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.

17. The recombinant or isolated polypeptide of claim 12 or 13 wherein the particle-associating portion of L polypeptide consists of an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or a functional derivative thereof comprising an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.

18. The recombinant or isolated polypeptide of claim 12 or 13 wherein the particle-associating portion of L-polypeptide comprises or consists essentially of amino acids 24 to 107 of SEQ ID NO: 9 or an amino acid sequence having at least 50% similarity thereto.

19. The recombinant or isolated polypeptide of claim 12 or 13 wherein said L polypeptide or particle-associating portion thereof is encoded by a sequence of

nucleotides substantially as set forth in SEQ ID NO: 6 or SEQ ID NO: 8 or having at least about 50% similarity to SEQ ID NO: 6 or SEQ ID NO: 8 or a contiguous sequence of nucleotides capable of hybridizing to a complementary form of SEQ ID NO: 6 or SEQ ID NO: 8 under hybridisation conditions of medium stringency.

20. The recombinant or isolated polypeptide according to claim 12 or 13 wherein said L polypeptide further comprises a signal sequence.

21. The recombinant or isolated polypeptide of claim 12 or 13 wherein the L polypeptide is a DHBV L polypeptide or a functional derivative thereof.

22. A recombinant nucleic acid molecule for use in making a VLP, said nucleic acid molecule comprising a contiguous sequence of nucleotides encoding a polypeptide of interest (POI) and at least a particle-associating portion of an L polypeptide of an avian hepadnavirus, such as DHBV, or a functional derivative thereof, wherein the POI is not a pre-S region of an avian hepadnavirus.

23. A recombinant nucleic acid molecule for use in making a VLP, said nucleic acid molecule comprising a contiguous sequence of nucleotides encoding i) a fusion polypeptide comprising a polypeptide of interest (POI) and at least a particle-associating portion of an L polypeptide of an avian hepadnavirus, such as DHBV, or a functional derivative thereof, and ii) a small envelope (S) polypeptide of an avian hepadnavirus such as DHBV or a functional derivative thereof, wherein the POI is not a pre-S region of an avian hepadnavirus.

24. The nucleic acid molecule of claim 22 or 23 wherein the particle-associating portion of L comprises at least the S domain of L, or the S domain of L minus the TM1 domain, or a functional derivative thereof.

25. The nucleic acid molecule of claim 22 or 23 wherein the POI is located in the pre-S domain of L or at the amino terminal side of the S domain of L, or the S domain

minus the TM1 domain of L.

26. The nucleic acid molecule of claim 22 or 23 which encodes an L polypeptide comprising an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or comprises an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.

27. The nucleic acid molecule of claim 26 which encodes a particle associating portion of L polypeptide consisting essentially of amino acids 24 to 167 of SEQ ID NO: 9 or an amino acid sequence having at least 50% similarity to SEQ ID NO: 9.

28. The nucleic acid molecule of claim 22 or 23 wherein said L polypeptide or particle associating portion thereof is encoded by a sequence of nucleotides substantially as set forth in SEQ ID NO: 6 or SEQ ID NO: 8 or having at least about 50% similarity to SEQ ID NO: 6 or SEQ ID NO: 8 or a contiguous sequence of nucleotides capable of hybridizing to a complementary form of SEQ ID NO: 6 or SEQ ID NO: 8 under hybridisation conditions of medium stringency.

29. The nucleic acid molecule of claim 22 or 23 wherein the L polypeptide further comprises a signal sequence.

30. The nucleic acid molecule of claim 22 or 23 wherein the L polypeptide is a DHBV L polypeptide or functional derivative thereof.

31. A recombinant nucleic acid molecule for use in making a VLP, said nucleic acid molecule encoding a particle-associating portion of an L polypeptide of an avian hepadnavirus, such as DHBV, or a functional derivative thereof and comprising one or more cloning sites suitable for accepting a nucleic acid molecule encoding a polypeptide of interest (POI), wherein said POI is expressed together with said L polypeptide and wherein the POI is not a pre-S region of an avian hepadnavirus.

32. A recombinant nucleic acid molecule for use in making a VLP, said nucleic acid molecule encoding at least a particle-associating portion of an L polypeptide of an avian hepadnavirus, such as DHBV, or a functional derivative thereof, and ii) a small envelope (S) polypeptide of an avian hepadnavirus, such as DHBV, or a functional derivative thereof and comprising one or more cloning sites suitable for accepting a nucleic acid molecule encoding a polypeptide of interest (POI), wherein said POI is expressed together with said L polypeptide and wherein the POI is not a pre-S region of an avian hepadnavirus.

33. The nucleic acid molecule of claim 31 or 32 wherein the particle-associating portion of L comprises at least the S domain of L, or the S domain of L minus the TM1 domain, or a functional derivative thereof.

34. The nucleic acid molecule of claim 31 or 32 wherein the POI is located in the pre-S domain of L or at the amino terminal side of the S domain of L, or the S domain minus the TM1 domain of L.

35. The nucleic acid molecule of claim 31 or 32 which encodes an L polypeptide comprising an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or comprises an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.

36. The nucleic acid molecule of claim 35 which encodes a particle-associating portion of L polypeptide consisting essentially of amino acids 24 to 167 of SEQ ID NO: 9 or an amino acid sequence having at least 50% similarity to SEQ ID NO: 9.

37. The nucleic acid molecule of claim 31 or 32 wherein said L polypeptide or particle-associating portion thereof is encoded by a sequence of nucleotides substantially as set forth in SEQ ID NO: 6 or SEQ ID NO: 8 or having at least about 50% similarity to SEQ ID NO: 6 or SEQ ID NO: 8 or a contiguous sequence of nucleotides capable of hybridizing to a complementary form of SEQ ID NO: 6 or SEQ ID NO:

8 under hybridisation conditions of medium stringency.

38. The nucleic acid molecule of claim 31 or 32 wherein the L polypeptide further comprises a signal sequence.

39. The nucleic acid molecule of claim 31 or 32 wherein the L polypeptide is a DHBV L polypeptide or functional derivative thereof.

40. An isolated and/or recombinant cell comprising the nucleic acid molecule of any one of claims 22 to 30 or expressing the polypeptide of any one of claims 12 to 21 or the VLP of any one of claims 1 to 11.

41. The cell according to claim 40 wherein said cell is a eukaryotic cell, preferably a yeast, avian or mammalian cell.

42. A method of delivering a POI to a subject or cell comprising expressing the POI in a VLP comprising L polypeptide from an avian hepadnavirus, such as DHBV, or a functional derivative thereof such that at least part of the POI is expressed on the surface of the VLP and administering the VLP to a subject or cell.

43. The method of claim 42 wherein the VLP is made in an *in vitro* expression system such as a yeast, avian or mammalian expression system.

44. The method of claim 42 wherein the VLP is made *in vivo* in the cells of a subject after administration of the nucleic acid molecule of any one of claims 22 to 30.

45. A method for making a recombinant VLP said method comprising:

i) cloning a nucleic acid molecule encoding a polypeptide of interest into an expression vector comprising a particle-associating portion of an L polypeptide of an avian hepadnavirus, such as DHBV, or a functional derivative thereof;

ii) introducing the recombinant expression vector of step i) into a suitable cell and maintaining same under conditions which allow protein expression and particle assembly with S polypeptide of an avian hepadnavirus, such as DHBV, or a functional derivative thereof; and

iii) recovering said virus-like particles from said cells.